
Names of God

A review of the expression and methylation pattern of *Wif1* and *WT1* genes ,in different types of cancers.

By :

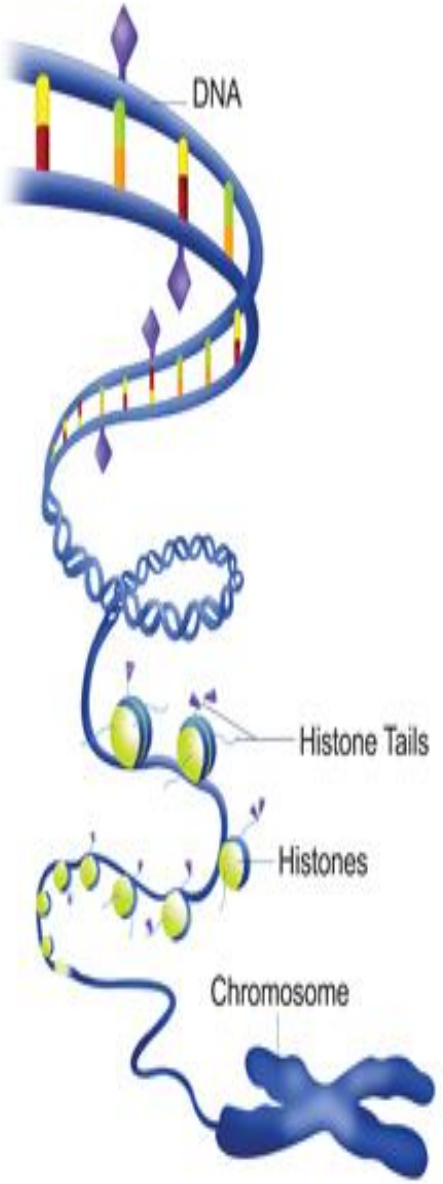
zahra mohammadi

Supervisor :

Dr. Gheibi

❑Content:

- ❖**Introduction**
- ✓Importance of Wnt signaling pathway
- ❖***Wif1***
- ❖***WT1***
- ❖**Epigenetic changes**
- ✓DNA Methylation
- ❖**Literature review**



Wnt signaling importance: (1)

- Is an ancient and evolutionarily conserved pathway that regulates crucial aspects of:
  - ✓ Cell fate determination.
  - ✓ Cell migration.
  - ✓ Organogenesis during embryonic development.
  - ✓ ….

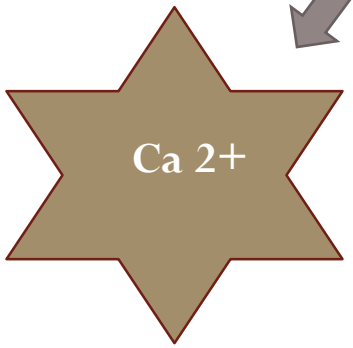
Wnt signaling pathways:

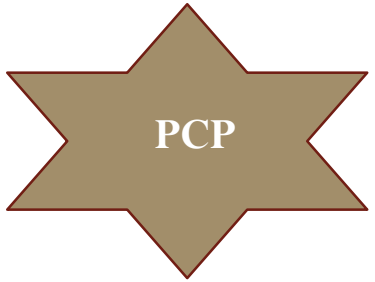
Including : (2)

1) **The canonical or Wnt/β-catenin dependent pathway**

2) **The non-canonical or β-catenin-independent pathway** which can be divided into the:

- ✓ Planar Cell Polarity pathway
- ✓ the Wnt/Ca2+ pathway





Osteoblast cell proliferation

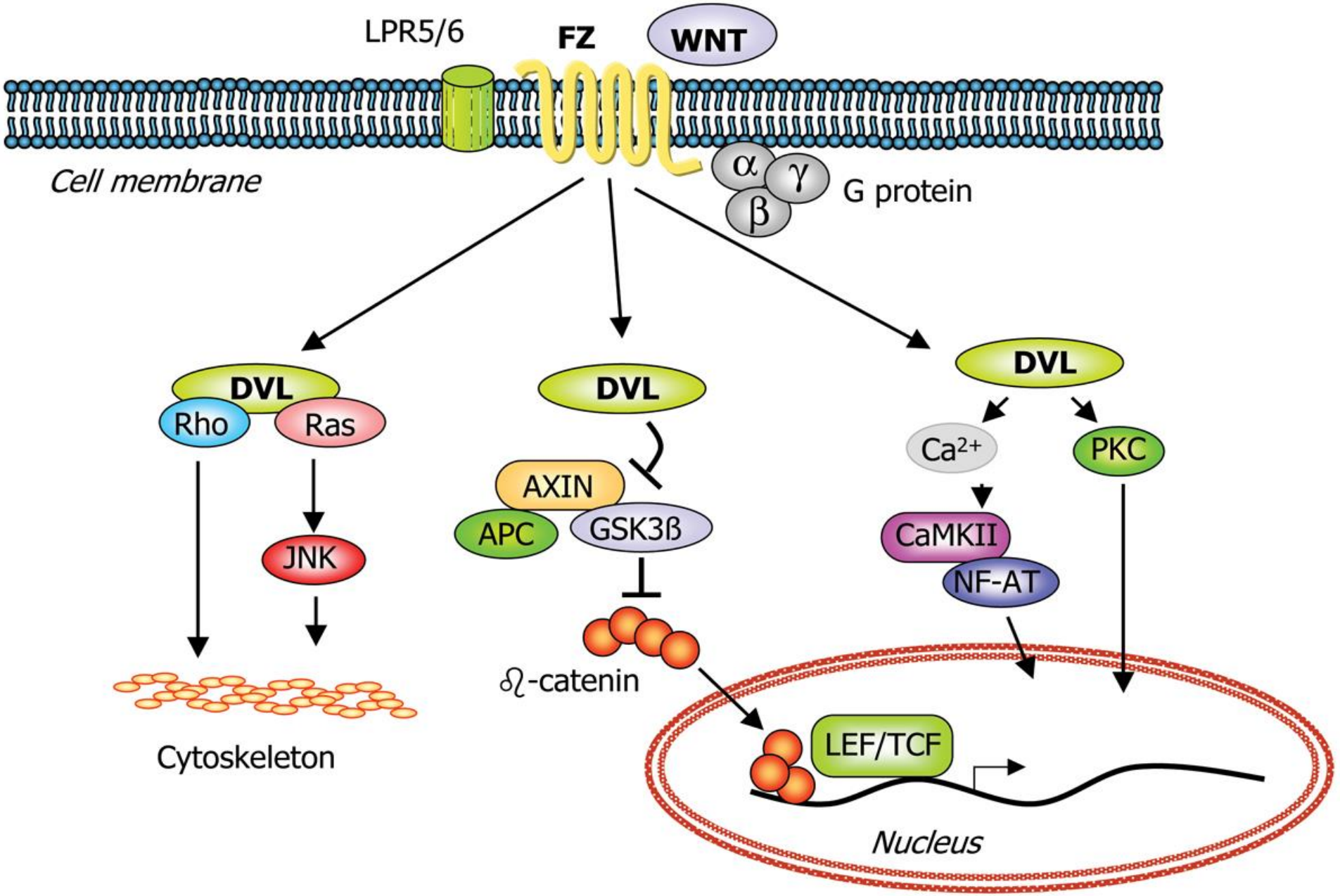
- Cell fate determination
- General bone maintenance
- Body axis specification
- **Cancer**

intracellular Ca2+ conc.

Activation of JNK

Affect cytoskeletal organisation

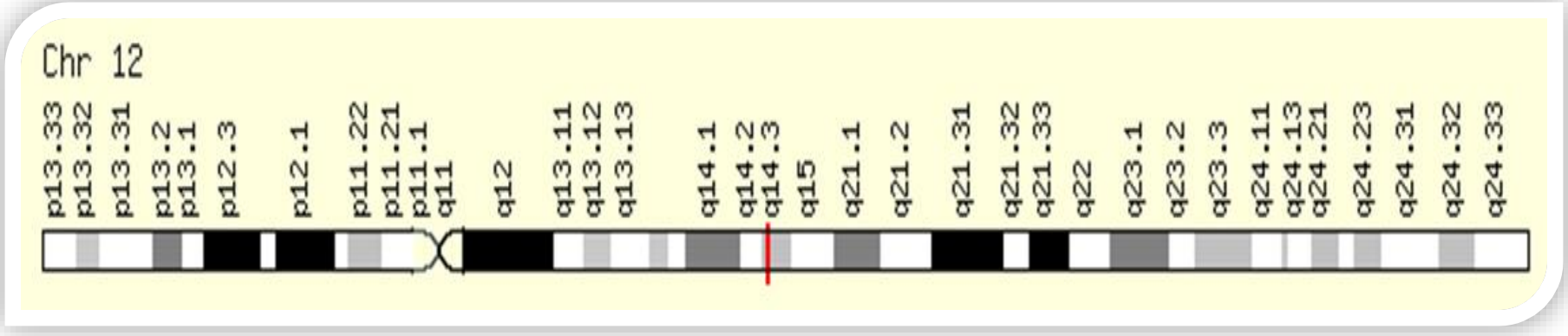
WNT signaling



WIF1

- Wnt inhibitory factor-1 , a secreted protein that binds to Wnt proteins and inhibits their activities. (3)

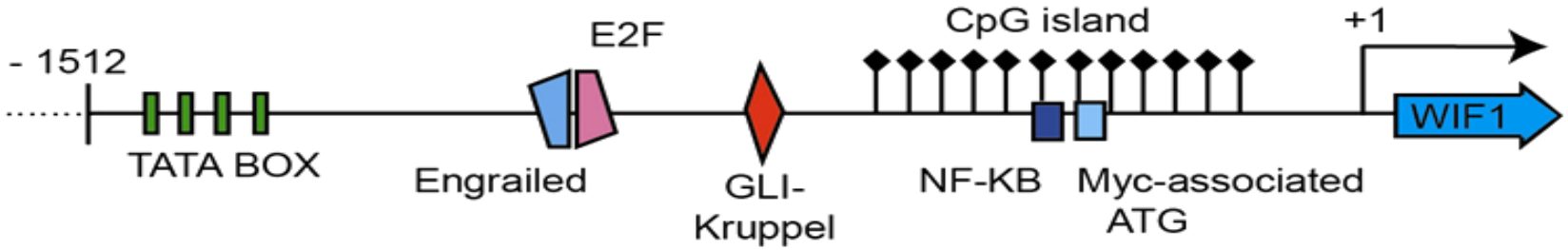
- WIF1 are potentially important in cancers as **Wnt pathway inhibitors** and **increased apoptosis**. (4)

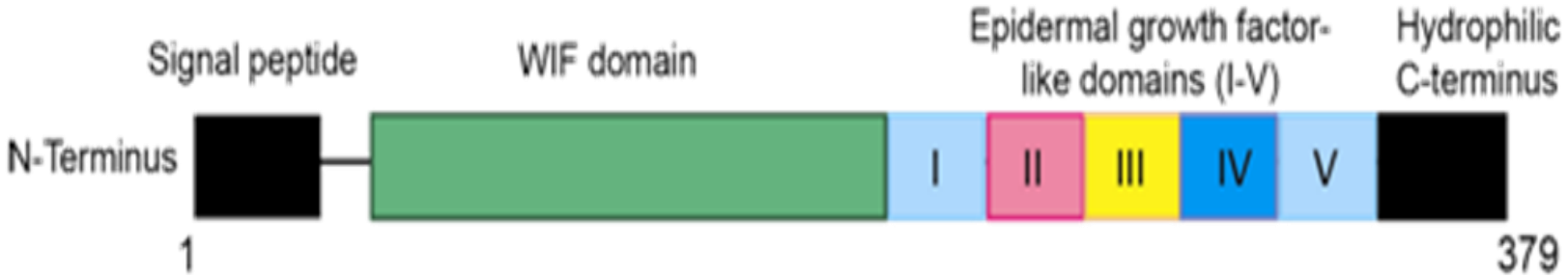


12q14.3

WIF1

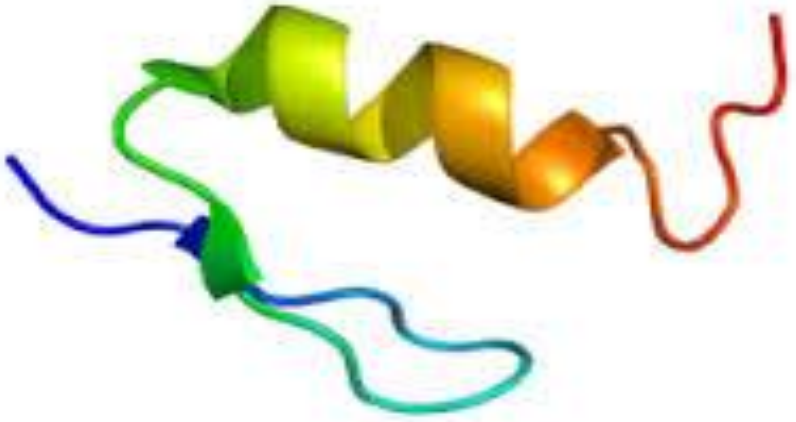
- This protein contains a WNT inhibitory factor (WIF) domain and 5 epidermal growth factor (EGF)-like domains. (5)



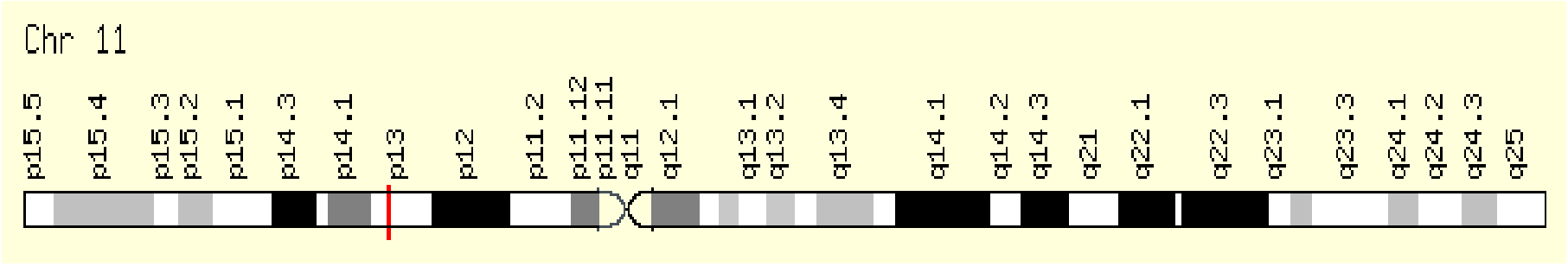


WT1

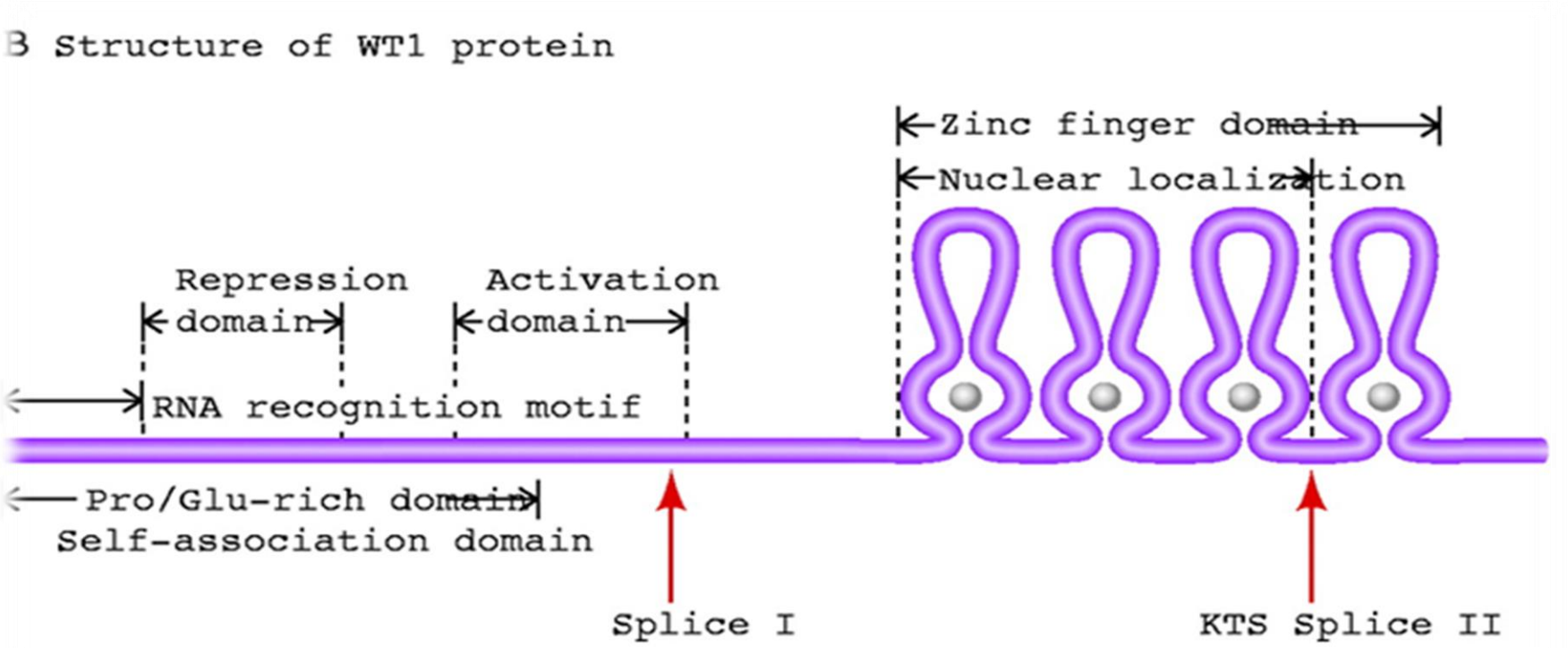
- 11p13
- 7490 bp



WT1 could be the role of the tumor suppressor or Oncogene. (6)



- WT1 encodes a zinc-finger transcription factor expressed in kidney, gonads, spleen, and mesothelium. (7)



GENETICS

mutations

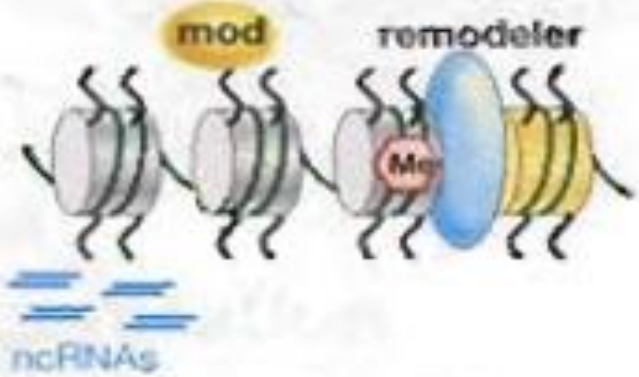
inherited

germ line

species

EPIGENETICS

alterations

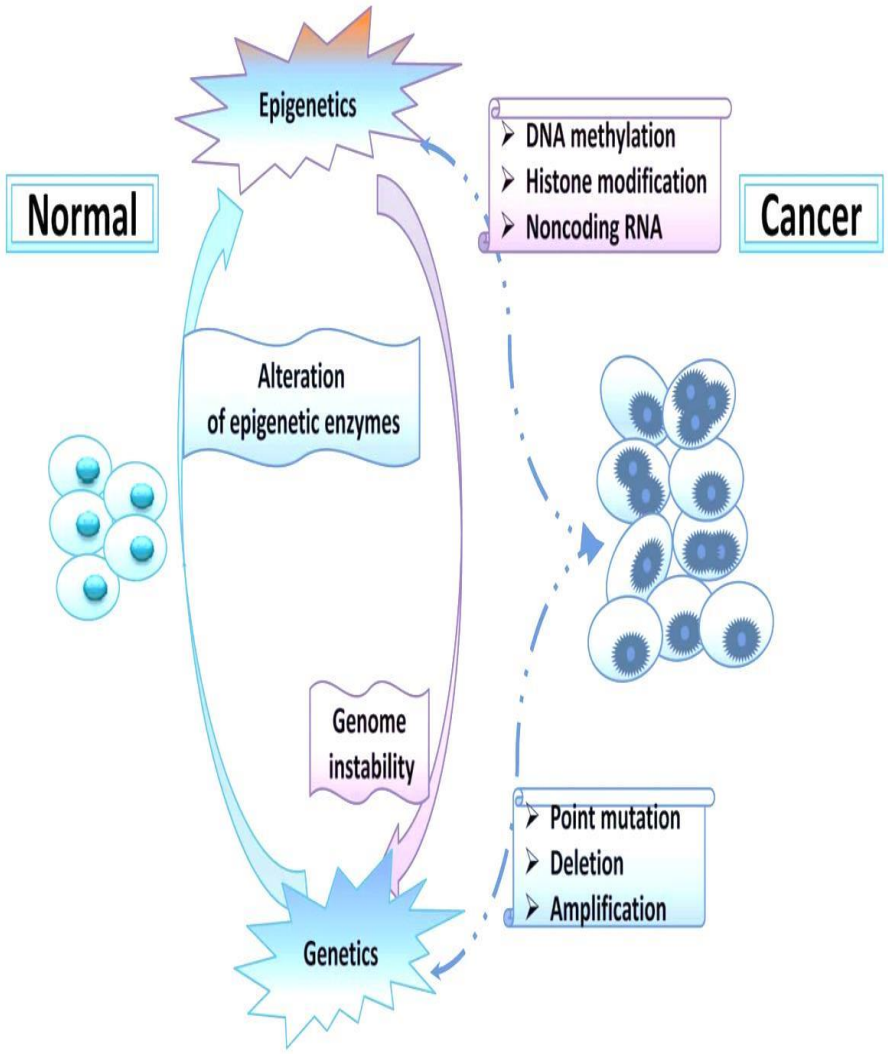


stable?

soma

variability

Defining Epigenetics



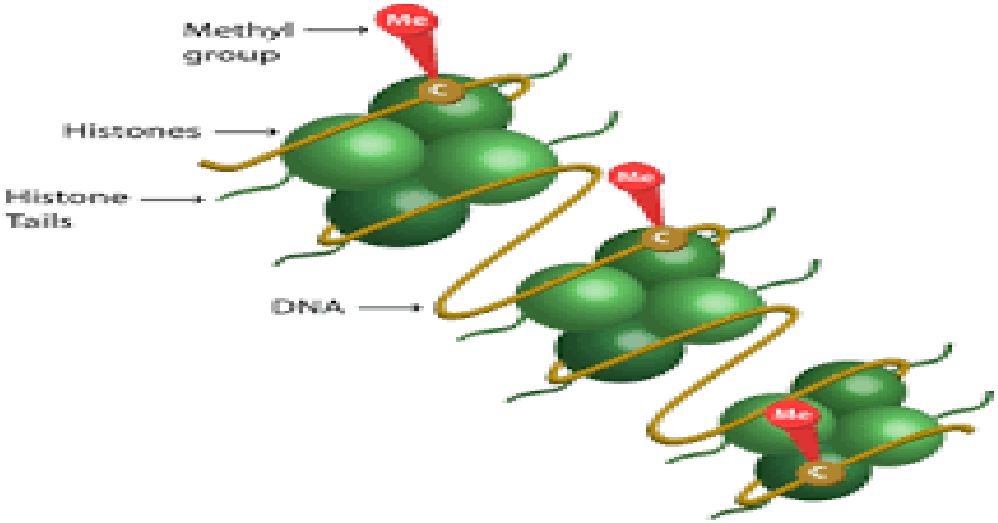
- Reversible changes in gene expression/function without changes in DNA sequence.
- Can be inherited from precursor cells. (8)

DNA methylation

- is an epigenetic marks associated with transcriptional regulation and genome structure. (9)

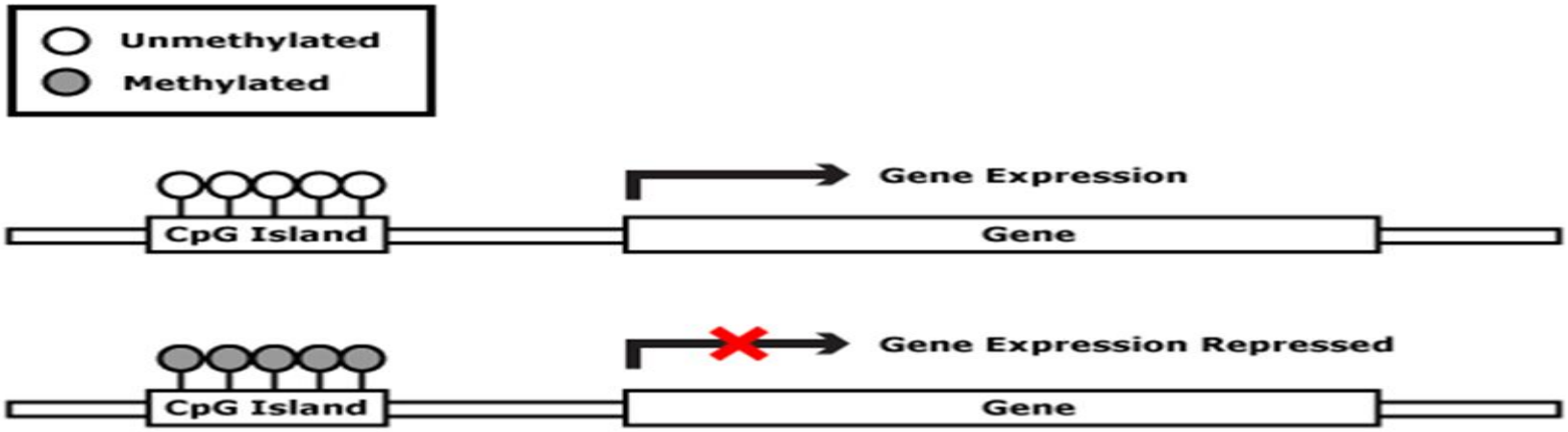
- Epigenetic changes in signaling pathway:

Wnt / B.catenin could be an attractive strategy for cancer therapy is dependent on the Wnt. (10)



- Methylation considered as a control mechanism of the gene profile while the activity of oncogenes cause development of tumor.

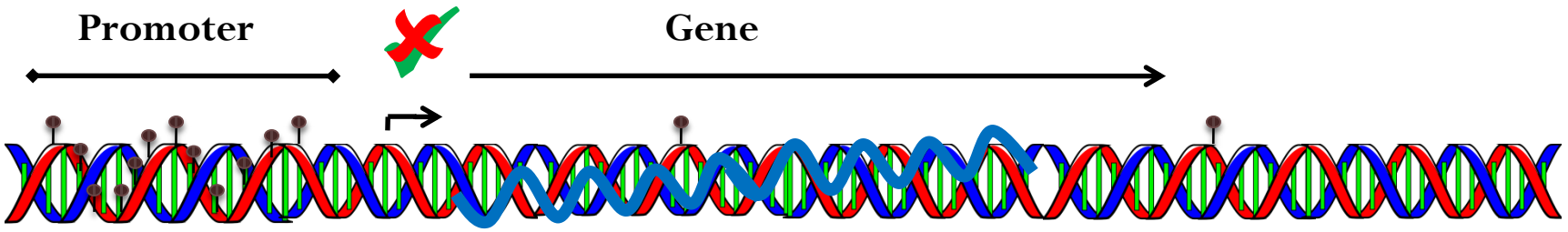
- Tumor suppressor factors play an important role in controlling and changing the genetic profile of terminally differentiated cells. (11)



What does DNA methylation do?

Global Methylation

Regional Methylation

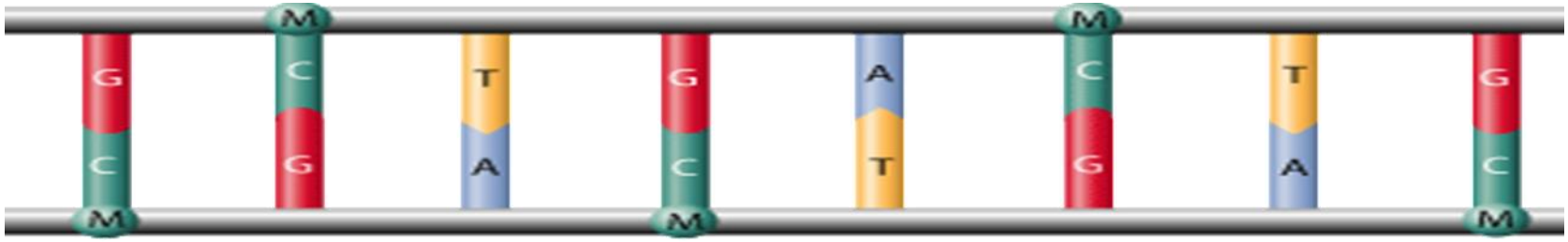


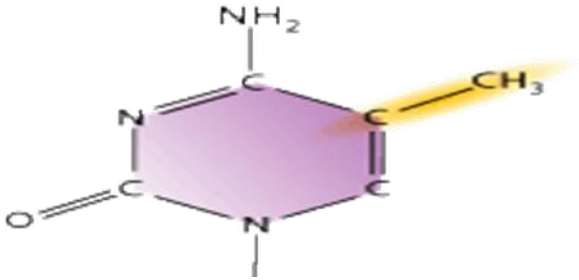
- High Density – CpG Islands (CGIs)
- Located in promoters and introns
- Gene Silencing

DNA Hypomethylation → activate oncogenes

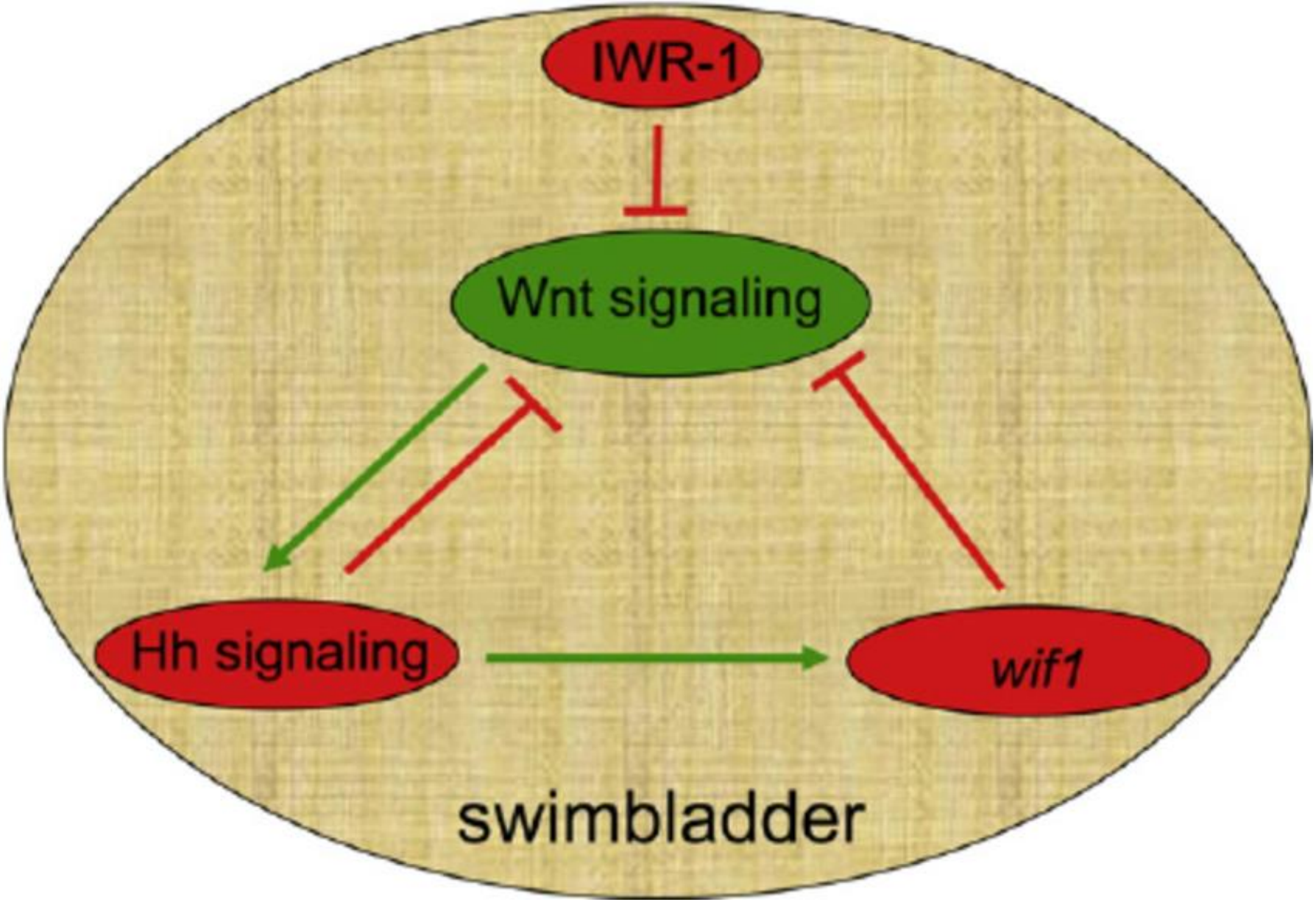
And initiate chromosome instability,

DNA Hypermethylation → silencing of tumor suppressor genes.





DNA methylation is the addition of a methyl group (M) to the DNA base cytosine (C).


IWR-1
Wnt signaling
Hh signaling
wif1
swimbladder

- ***Wif1*** antagonist of the Wnt pathway and is a **Tumor suppressor**. **(Epigenetic mechanisms in some tumors have been shown to cause inactivation of these genes.)** (12)
- Tumor suppressor gene **hypermethylation CPG** areas in many cases have been reported in human cancer. (including colorectal cancer) . (13)

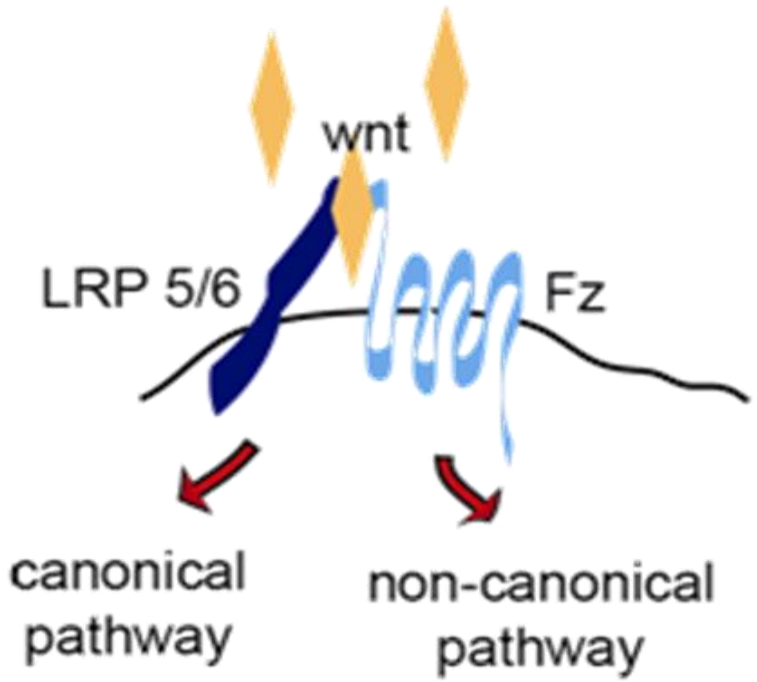
- **Decrease the Wnt pathway activity.**
- **Decrease cell growth.**
- **Increase cell apoptosis.** (14)

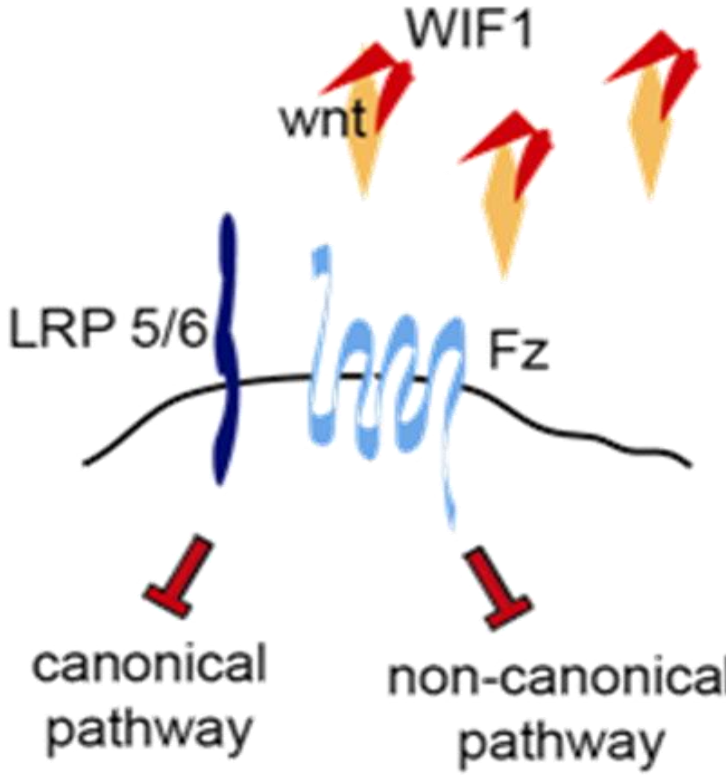
wif1

- ✓ is targeted for epigenetic silencing in human breast cancer. (15)

- ✓ ***Wif1*** methylation is a prognostic marker for cancer of the mouth. (16)

- ***Wif1*** gene in cancers of the prostate and breast, lung and bladder was **down regulated**. (9)





❑ Jaroslaw.et all ; 2015

Analyze methylation of ***Wif1*** in **16 OSCC cell line** and **43 tumor** section from primary OC patient, **frequently methylation** was shown. (9)

❑ Shanghui.et all ; 2015

Analyzed ***Wif1***-expression level by **IHC staining** and semi-quantitative **RT-PCR** was shown, Wif1 in normal oral mucous tissue was expressed, and in **OSCC** with progression of disease, **expression** has been **decreased**. (17)

❑ Ramachandran et al., 2011:

WIF1 is downregulated by promoter **hypermethylation** in 87.5% of primary **cervical cancer**. *(18)*

❑ Lambiv et al., 2011:

WIF1 re-expression abolishes tumorigenicity of *glioblastoma cell lines* potentially by **inducing senescence**. (19)

❑ Rubin et al., 2010:

Hypermethylation of the ***WIF1*** promoter is found in the majority of osteosarcoma cell lines tested and it correlates with WIF1 mRNA **down regulation.** (20)

❑ Deng et al., 2010:

WIF1 is frequently **down regulated** through promoter **hypermethylation** in HCC . (21)

❑ Kawakami et al., 2009:

WIF1 is downregulated by promoter **hypermethylation** and when re-expressed **induces apoptosis** in *Renal cell carcinoma (RCC)*cells. (22)

- ❖ **Bladder cancer** **(** Tang et al., 2009. 23 **)**
- ❖ **Breast cancer** **(** Ai et al., 2006. 24 **)**
- ❖ **Gastrointestinal cancers** **(** Taniguchi et al., 2005. 25 **)**

- ✓ **Reduction in regulatory** of ***WIF1*** examined in many malignant tumors such as:

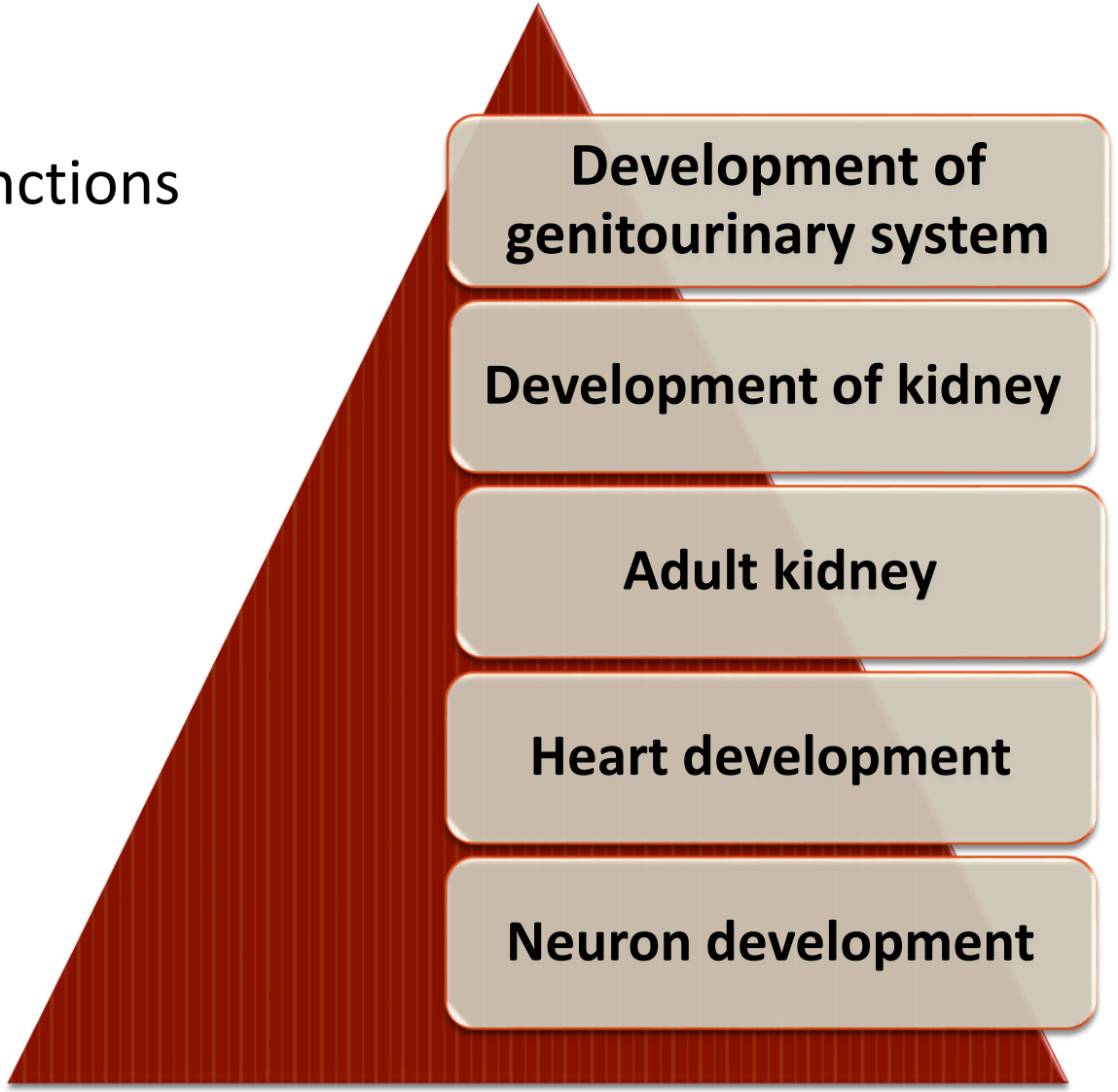
- ❑ Hepatocellular carcinoma Cancers . (26)
- ❑ Nasopharynx carcinoma Cancers . (27)
- ❑ Lung cancer. (28)
- ❑ Clone Cancers. (29)
- ❑ Esophageal adenocarcinoma Cancers . (30)
- ❑ Melanoma Cancers. (31)
- ❑ Bladder Cancers . (32)

- ✓ Decreased ***WIF1*** expression , correlated with methylation of CpG dinucleotides within the ***WIF1*** promoter. (33)

- ✓ Hypermethylation of ***WIF1*** gene promoter and low expression, activate Wnt signaling aberrantly and **induce** the development of **various human tumors**. (34)

WT1

Normal biological functions



- The Wilms' tumor 1 (WT1) gene encodes a transcription factor important for normal cellular development and cell survival. (35)

- WT1 was initially discovered as a tumor suppressor in Wilms' tumor

- The N terminus of WT1 is negatively charged and contains a high concentration of prolin and glutamine residues. (36)

WT1

- **Laux DE. et all ;1999**

detected aberrantly methylated sites in the **first intron** of the ***WT1*** gene in **breast cancer**. (37)

- **David M. et all ; 2001:**

WT1 is **overexpressed** in the majority of *primary* ***breast*** *tumors*. Tumor-specific methylation of the CpG island occurs in breast cancer but appears to be inconsequential to gene expression. (38)

❑ Oji Y.et all; 2003

In analyzed of **HNSCC** showed that ***WT1*** was not methylated. (39)

❑ Oji Y.et all ;2004

Overexpression of the ***WT1*** Gene in Esophageal Cancer. (40)

❑ Kaneuchi M.et all ;2005

examined The ***WT1*** promoter was significantly **methylated** in clear cell **adenocarcinoma** (88.2%) compared with serous adenocarcinoma (24.0%). (41)

❑ Moriya S.et all; 2008

Showed that , in **lung SCC** ,***WT1*** expression was **down regulated**. (42)

❑ Pierdonato B. et al., 2012

Hypermethylation of ***WT1*** gene could be involved in the carcinogenesis of **NSCLC** (non small cell lung cancer).

❑ Jitesh PV.et all; 2013

WT1 gene in **ESCC** and **Non ESCC** patient has different methylation status.

And show that, epigenetic changes Necessary for cancer development. (43)

- **MikamiyT.et all; 2013**

In analyze of **6 cell line of OSCC** with IHC , ***WT1*** was **over expressed**. (44)

- **Shimodaria S.et all; 2016**

WT1 **over expression** was observed in autopsy sample of **pancreatic** adenocarcinoma. (45)

- **Oji Y.et all; 2016**

In leukemia ***WT1*** have high expression. (46)

offer

- **Methylation of genes as biomarkers for cancer detection model can be used.**

- **Furthermore, by recognizing the methylation patterns in cancer types, it can also be used for treatment.**


Thank you!

- **References:**

1. Komiya Y, Habas R. Wnt signal transduction pathways. Organogenesis. 2008;4(2):68-75.

2. Rao TP, Kühl M. An updated overview on Wnt signaling pathways a prelude for more. Circulation research. 2010;106(12):1798-806.

3. Hsieh JC, Kodjabachian L, Rebbert ML, Rattner A, Smallwood PM, Samos CH, et al. A new secreted protein that binds to Wnt proteins and inhibits their activities. Nature. 1999;398(6726):431-6.

4. Stewart DJ, Chang DW, Ye Y, Spitz M, Lu C, Shu X, et al. Wnt signaling pathway pharmacogenetics in non-small cell lung cancer. The pharmacogenomics journal. 2014;14(6):509-22.

5. Muegge K, Geiman T. HELLS (Helicase, Lymphoid-Specific). 2014.

6. Loeb DM, Evron E, Patel CB, Sharma PM, Niranjan B, Buluwela L, et al. Wilms' tumor suppressor gene (WT1) is expressed in primary breast tumors despite tumor-specific promoter methylation. Cancer Research. 2001;61(3):921-5.

7. Haber DA, Sohn RL, Buckler AJ, Pelletier J, Call KM, Housman DE. Alternative splicing and genomic structure of the Wilms tumor gene WT1. Proceedings of the National Academy of Sciences. 1991;88(21):9618-22

- 8. Verma M, Srivastava S. Epigenetics in cancer: implications for early detection and prevention. The lancet oncology. 2002;3(12):755-63.
- 9. Brennan K. Blood DNA methylation biomarkers for breast cancer risk. 2013.
- 10. Ying Y, Tao Q. Epigenetic disruption of the WNT/ß-catenin signaling pathway in human cancers. Epigenetics. 2009;4(5):307-12.
- 11. Azad M, Kaviani S, Noruzinia M, Mortazavi Y, Mobarra N, Alizadeh S, et al. Gene Expression Status and Methylation Pattern in Promoter of P15INK4b and P16INK4a in Cord Blood CD34+ Stem Cells. Iranian journal of basic medical sciences. 2013;16(7):822.
- 12. Chan SL, Cui Y, Van Hasselt A, Li H, Srivastava G, Jin H, et al. The tumor suppressor Wnt inhibitory factor 1 is frequently methylated in nasopharyngeal and esophageal carcinomas. Laboratory investigation. 2007;87(7):644-50.
- 13. Lee BB, Lee EJ, Jung EH, Chun H-K, Chang DK, Song SY, et al. Aberrant methylation of APC, MGMT, RASSF2A, and Wif-1 genes in plasma as a biomarker for early detection of colorectal cancer. Clinical Cancer Research. 2009;15(19):6185-91.
- 14. Logan CY, Nusse R. The Wnt signaling pathway in development and disease. Annu Rev Cell Dev Biol. 2004;20:781-810.

- 15. Ai L, Tao Q, Zhong S, Fields CR, Kim W-J, Lee MW, et al. Inactivation of Wnt inhibitory factor-1 (WIF1) expression by epigenetic silencing is a common event in breast cancer. Carcinogenesis. 2006;27(7):1341-8.
- 16. Paluszczak J, Sarbak J, Kostrzewska-Poczekaj M, Kiwerska K, Jarmuż-Szymczak M, Grenman R, et al. The negative regulators of Wnt pathway—DACH1, DKK1, and WIF1 are methylated in oral and oropharyngeal cancer and WIF1 methylation predicts shorter survival. Tumor Biology. 2015;36(4):2855-61.
- 17. Zhou S, Chen L, Mashrah M, Zhu Y, He Z, Hu Y, et al. Expression and promoter methylation of Wnt inhibitory factor-1 in the development of oral submucous fibrosis. Oncology reports. 2015;34(5):2636-42.
- 18. Ramachandran I, Reis AM, Anant S, Queimado L, Thavathiru E, Ramalingam S, et al. Abstract C21: Wnt inhibitory factor 1 is silenced by promoter hypermethylation in cervical cancer and its restoration suppresses tumor growth. Cancer Research. 2011;71(18 Supplement):C21-C.
- 19. Hegi ME, Diserens A-C, Bady P, Kamoshima Y, Kouwenhoven MC, Delorenzi M, et al. Pathway analysis of glioblastoma tissue after preoperative treatment with the EGFR tyrosine kinase inhibitor gefitinib—a phase II trial. Molecular cancer therapeutics. 2011;10(6):1102-12.
- 20. Rubin EM, Guo Y, Tu K, Xie J, Zi X, Hoang BH. Wnt inhibitory factor 1 decreases tumorigenesis and metastasis in osteosarcoma. Molecular cancer therapeutics. 2010;9(3):731-41.
- 21. Deng Y, Yu B, Cheng Q, Jin J, You H, Ke R, et al. Epigenetic silencing of WIF-1 in hepatocellular carcinomas. Journal of cancer research and clinical oncology. 2010;136(8):1161-7.

- 22. Kawakami K, Hirata H, Yamamura S, Kikuno N, Saini S, Majid S, et al. Functional significance of Wnt inhibitory factor-1 gene in kidney cancer. Cancer research. 2009;69(22):8603-10.
- 23. Tang Y, Simoneau AR, Liao W-x, Yi G, Hope C, Liu F, et al. WIF1, a Wnt pathway inhibitor, regulates SKP2 and c-myc expression leading to G1 arrest and growth inhibition of human invasive urinary bladder cancer cells. Molecular cancer therapeutics. 2009;8(2):458-68.
- 24. Ai L, Tao Q, Zhong S, Fields CR, Kim W-J, Lee MW, et al. Inactivation of Wnt inhibitory factor-1 (WIF1) expression by epigenetic silencing is a common event in breast cancer. Carcinogenesis. 2006;27(7):1341-8.
- 25. Taniguchi H, Yamamoto H, Hirata T, Miyamoto N, Oki M, Nosho K, et al. Frequent epigenetic inactivation of Wnt inhibitory factor-1 in human gastrointestinal cancers. Oncogene. 2005;24(53):7946-52.
- 26. Huang L, Li M-X, Wang L, Li B-K, Chen G-H, He L-R, et al. Prognostic value of Wnt inhibitory factor-1 expression in hepatocellular carcinoma that is independent of gene methylation. Tumor Biology. 2011;32(1):233-40.
- 27. Fendri A, Khabir A, Hadri-Guiga B, Sellami-Boudawara T, Daoud J, Frikha M, et al. Epigenetic alteration of the Wnt inhibitory factor-1 promoter is common and occurs in advanced stage of Tunisian nasopharyngeal carcinoma. Cancer investigation. 2010;28(9):896-903.

- 28. Yoshino M, Suzuki M, Tian L, Moriya Y, Hoshino H, Okamoto T, et al. Promoter hypermethylation of the p16 and Wif-1 genes as an independent prognostic marker in stage IA non-small cell lung cancers. International journal of oncology. 2009;35(5):1201
- 29. Lee BB, Lee EJ, Jung EH, Chun H-K, Chang DK, Song SY, et al. Aberrant methylation of APC, MGMT, RASSF2A, and Wif-1 genes in plasma as a biomarker for early detection of colorectal cancer. Clinical Cancer Research. 2009;15(19):6185-91
- 30. Clément G, Guilleret I, He B, Yagui-Beltrán A, Lin YC, You L, et al. Epigenetic alteration of the Wnt inhibitory factor-1 promoter occurs early in the carcinogenesis of Barrett's esophagus. Cancer science. 2008;99(1):46-53.
- 31. Lin Y-C, You L, Xu Z, He B, Yang C-T, Chen J-K, et al. Wnt inhibitory factor-1 gene transfer inhibits melanoma cell growth. Human gene therapy. 2007;18(4):379-86
- 32. Urakami S, Enokida H, Ogishima T, Kawakami T, Tokizane T, Tanaka Y, et al. Epigenetic inactivation of Wnt inhibitory factor-1 plays an important role in bladder carcinogenesis through aberrant Wnt signaling. Cancer Research. 2005;65(9 Supplement):430.
- 33. Qi J, Zhu Y, Luo J, Tao W. [The role of secreted Wnt-antagonist genes hypermethylation in early detection of colorectal tumor]. Zhonghua yi xue za zhi. 2007;87(28):1954-7.

- 34. Yang Z, Wang Y, Fang J, Chen F, Liu J, Wu J, et al. Expression and aberrant promoter methylation of Wnt inhibitory factor-1 in human astrocytomas. Journal of Experimental & Clinical Cancer Research. 2010;29(1):1.
- 35. Yang L, Han Y, Saiz FS, Minden M. A tumor suppressor and oncogene: the WT1 story. Leukemia. 2007;21(5):868-76.
- 36. Wang Z, Qiu Q-Q, Deuel T. The Wilms' tumor gene product WT1 activates or suppresses transcription through separate functional domains. Journal of Biological Chemistry. 1993;268(13):9172-5.
- 37. Laux DE, Curran EM, Welshons WV, Lubahn DB, Huang THM. Hypermethylation of the Wilms' tumor suppressor gene CpG island in human breast carcinomas. Breast cancer research and treatment. 1999;56(1):35-43.
- 38. Loeb DM, Evron E, Patel CB, Sharma PM, Niranjan B, Buluwela L, et al. Wilms’ tumor suppressor gene (WT1) is expressed in primary breast tumors despite tumor-specific promoter methylation. Cancer Research. 2001;61(3):921-5.
- 39. Oji Y, Inohara H, Nakazawa M, Nakano Y, Akahani S, Nakatsuka Si, et al. Overexpression of the Wilms' tumor gene WT1 in head and neck squamous cell carcinoma. Cancer science. 2003;94(6):523-9.

- 40. OJI Y, YANO M, NAKANO Y, ABENO S, NAKATSUKA S-I, IKEBA A, et al. Overexpression of the Wilms' tumor gene WT1 in esophageal cancer. Anticancer research. 2004;24(5B):3103-8.
- 41. Kaneuchi M, Sasaki M, Tanaka Y, Shiina H, Yamada H, Yamamoto R, et al. WT1 and WT1-AS genes are inactivated by promoter methylation in ovarian clear cell adenocarcinoma. Cancer. 2005;104(9):1924-30.
- 42. Moriya S, Takiguchi M, Seki N. Expression of the WT1 gene-KTS domain isoforms suppresses the invasive ability of human lung squamous cell carcinoma cells. International journal of oncology. 2008;32(2):349-56.
- 43. Jithesh P, Risk J, Schache A, Dhanda J, Lane B, Liloglou T, et al. The epigenetic landscape of oral squamous cell carcinoma. British journal of cancer. 2013;108(2):370-9.
- 44. Mikami T, Hada T, Chosa N, Ishisaki A, Mizuki H, Takeda Y. Expression of Wilms’ tumor 1 (WT1) in oral squamous cell carcinoma. Journal of Oral Pathology & Medicine. 2013;42(2):133-9.
- 45. Shimodaira S, Hirabayashi K, Koya T, Higuchi Y, Sano K. Standard WT1 Expression Using Immunohistochemistry. Journal of Molecular Biomarkers & Diagnosis. 2016;2016.

- 46. Oji Y, Hashimoto N, Tsuboi A, Murakami Y, Iwai M, Kagawa N, et al. Association of WT1 IgG antibody against WT1 peptide with prolonged survival in glioblastoma multiforme patients vaccinated with WT1 peptide. International journal of cancer. 2016.